CLAIMS

The invention claimed is:

1. A chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide.

2. The chimeric protein of Claim 1, wherein the domains are linked by at least one amino acid.

3. The chimeric protein of Claim 2, wherein at least one of the domains is selected from the group consisting of the homeodomain DNA-binding domains.

4. The chimeric protein of Claim 3, wherein the domain is the Oct-1 homeodomain.

5. The chimeric protein of Claim 2, wherein at least one of the domains is selected from the group consisting of the zinc finger DNA-binding domains.

6. The chimeric protein of Claim 5, wherein the zinc finger DNA-binding domain is finger 1 or finger 2 of Zif268.

7. A chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least three domains, referred to as the first domain, the second domain and the third domain, each of which is a DNA-binding polypeptide which binds a sequence which

is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain and the carboxyl-terminal region of the second domain is linked to the amino-terminal region of the third domain to form a continuous polypeptide, such that the chimeric protein binds a composite DNA sequence.

8. The chimeric protein of Claim 7, wherein the first domain is linked to the second domain by at least one amino acid or the second domain is linked to the third domain by at least one amino acid.

9. A transcription factor which regulates gene expression in a cell, said transcription factor comprising:

a) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

b) at least one activation domain.

10. The transcription factor of Claim 9, wherein the activation domain is the Herpes Simplex Virus VP16 activation domain.

11. The transcription factor of Claim 9, wherein the domains are linked by at least one amino acid.

12. The transcription factor of Claim 10, wherein at least one of the domains is selected from the group consisting of the homeodomain DNA-binding domains.

13. The transcription factor of Claim 12, wherein the homeodomain is the Oct-1 homeodomain.

14. The transcription factor of Claim 10, wherein at least one of the domains is selected from the group consisting of the zinc finger DNA-binding domains.

15. The transcription factor of Claim 14, wherein the zinc finger DNA-binding domain is zinc finger 1 or zinc finger 2 of Zif268.

16. A transcription factor which regulates gene expression in a cell, said transcription factor comprising:

a) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises the Oct-1 homeodomain and zinc fingers 1 and 2 of Zif268, and wherein the carboxyl-terminal region of zinc finger 2 is linked to the amino terminal region of the Oct-1 homeodomain by at least one amino acid to form a continuous polypeptide; and

b) the Herpes Simplex Virus VP16 activation domain.

17. A transcription factor which regulates gene expression in a cell, said transcription factor comprising:

a) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first

domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

b) at least one repression domain.

18. A transcription factor which regulates gene expression in a cell, said transcription factor comprising a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain.

19. A chimeric cleavage protein which cleaves DNA, said cleavage protein comprising:

a) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

b) at least one DNA cleavage domain.

20. The chimeric cleavage protein of Claim 19, wherein the domains are linked by at least one amino acid.

21. The chimeric cleavage protein of Claim 20, wherein the DNA cleavage domain is the FokI cleavage domain.

22. An expression construct comprising DNA encoding the chimeric protein of Claim 2.

23. An expression construct comprising DNA encoding the transcription factor of Claim 10.

24. An expression construct comprising DNA encoding the chimeric cleavage protein of Claim 20.

25. An expression construct comprising DNA encoding the transcription factor of Claim 17.

26. An expression construct comprising DNA encoding the transcription factor of Claim 18.

27. A method of constructing a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide, said method comprising the steps of:

a) using a modeling system to superimpose two structures, each of which is a DNA-binding polypeptide bound to DNA, upon one another in various arrangements;

b) determining which DNA-binding polypeptides may be combined to form a chimeric protein;

c) joining the DNA-binding polypeptides of step b) to create a chimeric protein, such that the DNA-binding polypeptides retain the ability to bind their respective DNA sequences, and such that the

carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

e) assessing the binding specificity of the chimeric protein,

thereby constructing a chimeric protein which binds a composite DNA sequence.

28. The method of Claim 27, wherein the domains are linked by one or more amino acids.

29. A method of identifying a specific DNA sequence in a mixture, comprising the steps of:

a) selecting a DNA sequence to be identified in a mixture;

b) constructing a chimeric protein which binds the selected DNA sequence of a); and

c) introducing the chimeric protein into the mixture under conditions appropriate for specific binding of the chimeric protein to the DNA sequence,

wherein specific binding of the chimeric protein identifies the DNA sequence.

30. The method of Claim 29, wherein the chimeric protein is labelled with an affinity ligand.

31. A method of positively regulating the transcription of a gene in a cell comprising the steps of:

a) obtaining a transcription factor which binds a DNA sequence in a regulatory element of a gene, said transcription factor comprising:

1) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of

which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

2) at least one activation domain; and

b) expressing the transcription factor of a) in a cell containing the gene,

whereby the transcription factor binds the DNA sequence and activates transcription of the gene, thereby positively regulating transcription of the gene.

32. The method of Claim 31, wherein expression of the transcription factor is controlled by an inducible promoter.

33. A method of inhibiting transcription of a gene in a cell, comprising the steps of:

a) introducing an expression construct comprising DNA encoding the chimeric protein of Claim 2 into a cell such that a stably transformed cell is obtained; and

b) expressing the DNA segment encoding the chimeric protein,

whereby the chimeric protein specifically binds to the gene and inhibits transcription.

34. A method of cleaving DNA <u>in vitro</u>, comprising:

a) obtaining a chimeric cleavage protein which recognizes a DNA sequence and cleaves DNA adjacent to said sequence, said cleavage protein comprising:

1) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

2) at least one DNA cleavage domain; and

b) expressing the cleavage protein of a), whereby the cleavage protein binds the composite DNA sequence and cleaves the DNA adjacent to said composite DNA sequence.

35. A method of gene therapy for increasing the expression of a deficient gene product, comprising

a) identifying a cellular gene responsible for deficient production of a gene product;

b) obtaining a transcription factor which binds a DNA sequence in a regulatory element of the gene, said transcription factor comprising:

1) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

2) at least one activation domain;

c) introducing the transcription factor of step b) into a cell containing the gene of step a); and

d) expressing the transcription factor of step c), thereby increasing the expression of a deficient gene product.

36. A method of constructing a chimeric protein which binds a selected composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide, said method comprising the steps of:

a) selecting a composite DNA sequence for recognition by a chimeric protein;

b) identifying DNA-binding polypeptides which bind to portions of the composite sequence;

c) using a modeling system to determine how the DNA-binding polypeptides of step b) can be linked to form a chimeric protein;

d) joining the DNA-binding polypeptides of step c) to create a chimeric protein, such that the DNA-binding polypeptides retain the ability to bind their respective DNA sequences, and such that the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide,

thereby constructing a chimeric protein which binds the selected composite DNA sequence.

37. A method of negatively regulating the expression of a gene in a cell comprising the steps of:

a) obtaining a transcription factor which binds a DNA sequence in a regulatory element of a gene, said transcription factor comprising:

1) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

2) at least one repression domain; and

b) expressing the transcription factor of a) in a cell containing the gene,

whereby the transcription factor binds the DNA sequence and negatively regulates transcription of the gene, thereby negatively regulating expression of the gene.

38. A method of gene therapy for decreasing the expression of a gene product, comprising

a) identifying a gene responsible for the expression of the selected gene product;

b) obtaining a transcription factor which binds a DNA sequence in a regulatory element of the gene, said transcription factor comprising:

1) a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of

which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide; and

2) at least one repression domain;

c) introducing the transcription factor of step b) into a cell containing the gene of step a); and

d) expressing the transcription factor of step c), whereby the transcription factor binds a DNA sequence in the regulatory element of the gene, thereby decreasing the expression of a gene product.

39. A method of gene therapy for decreasing the expression of a gene product, comprising

a) identifying a cellular gene responsible for the expression of the selected gene product;

b) obtaining a transcription factor which binds a DNA sequence in a regulatory element of the gene, said transcription factor comprising a chimeric protein which binds a composite DNA sequence, wherein said protein comprises at least two domains, referred to as the first domain and the second domain, each of which is a DNA-binding polypeptide which binds a sequence which is a portion of the composite DNA sequence, and wherein the carboxyl-terminal region of the first domain is linked to the amino-terminal region of the second domain to form a continuous polypeptide;

c) introducing the transcription factor of step b) into a cell containing the gene of step a); and

d) expressing the transcription factor of step c),

whereby the transcription factor binds a DNA sequence in the regulatory element of the gene, thereby decreasing the expression of a gene product.